

**METHODS AND REAGENTS FOR MOLECULAR CLONING**

**ABSTRACT OF THE INVENTION**

The present invention provides compositions, methods, and kits for covalently linking nucleic acid molecules. The methods include a strand invasion step, and the compositions and kits are useful for performing such methods. For example, a method of covalently linking double stranded (ds) nucleic acid molecules can include contacting a first ds nucleic acid molecule, which has a topoisomerase linked to a 3' terminus of one end and has a single stranded 5' overhang at the same end, with a second ds nucleic acid molecule having a blunt end, such that the 5' overhang can hybridize to a complementary sequence of the blunt end of the second nucleic acid molecule, and the topoisomerase can covalently link the ds nucleic acid molecules. The methods are simpler and more efficient than previous methods for covalently linking nucleic acid sequences, and the compositions and kits facilitate practicing the methods, including methods of directionally linking two or more ds nucleic acid molecules.